Coagulation Disturbances in Patients with Argininemia

Kiykim, Ertugrul ; Zubarioglu, Tanyel ; Cansever, Mehmet Serif ; Celkan, Tiraje ; Häberle, Johannes ; Aktuglu Zeybek, Ayse Cigdem

Abstract: BACKGROUND Argininemia is an autosomal recessive urea cycle disorder (UCD). Unlike other UCD, hyperammonemia is rarely seen. Patients usually present in childhood with neurological symptoms. Uncommon presentations like neonatal cholestasis or cirrhosis have been reported. Although transient elevations of liver transaminases and coagulopathy have been reported during hyperammonemia episodes, a permanent coagulopathy has never been reported. METHODS In this retrospective study, coagulation disturbances are examined in 6 argininemia patients. All of the patients were routinely followed up for hepatic involvement due to argininemia. Laboratory results, including liver transaminases, albumin, prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), and clotting factor levels, were assessed in all of the patients. RESULTS All of the patients had a prolonged PT and an increased INR, while none of the patients had a prolonged aPTT. Five patients had slightly elevated liver transaminases. A liver biopsy was performed in 1 patient but neither cirrhosis nor cholestasis was documented. Five of the 6 patients had low factor VII and factor IX levels, while other clotting factors were normal. CONCLUSIONS Argininemia patients should be investigated for coagulation disorders even if there is no apparent liver dysfunction or major bleeding symptoms.

DOI: https://doi.org/10.1159/000493678

Original Paper

Acta Hæmatologica

Acta Haematol 2018;140:221–225
DOI: 10.1159/000493678

Received: January 8, 2018
Accepted after revision: September 6, 2018
Published online: October 24, 2018

Coagulation Disturbances in Patients with Argininemia

Ertugrul Kiykim[a] Tanyel Zubarioglu[a] Mehmet Serif Cansever[b] Tiraje Celkan[c] Johannes Häberle[d] Ayse Cigdem Aktuglu Zeybek[a]

[a]Division of Nutrition and Metabolism, Department of Pediatrics, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey; [b]Central Laboratory, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey; [c]Division of Hematology and Oncology, Department of Pediatrics, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey; [d]Division of Metabolism, University Children's Hospital, Zurich, Switzerland

Keywords
Argininemia · Coagulation disorders · Factor deficiency · Inherited metabolic disorder

Abstract
Background: Argininemia is an autosomal recessive urea cycle disorder (UCD). Unlike other UCD, hyperammonemia is rarely seen. Patients usually present in childhood with neurological symptoms. Uncommon presentations like neonatal cholestasis or cirrhosis have been reported. Although transient elevations of liver transaminases and coagulopathy have been reported during hyperammonemia episodes, a permanent coagulopathy has never been reported. ***Methods:*** In this retrospective study, coagulation disturbances are examined in 6 argininemia patients. All of the patients were routinely followed up for hepatic involvement due to argininemia. Laboratory results, including liver transaminases, albumin, prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), and clotting factor levels, were assessed in all of the patients. ***Results:*** All of the patients had a prolonged PT and an increased INR, while none of the patients had a prolonged aPTT. Five patients had slightly elevated liver transaminases. A liver biopsy was performed in 1 patient but neither cirrhosis nor cholestasis was documented. Five of the 6 patients had low factor VII and factor IX levels, while other clotting factors were normal. ***Conclusions:*** Argininemia patients should be investigated for coagulation disorders even if there is no apparent liver dysfunction or major bleeding symptoms.

Introduction

Argininemia is an autosomal recessive disease caused by a defect in arginase 1 (ARG1), an enzyme involved in the urea cycle. Three urea cycle enzymes, i.e., N-acetylglutamate synthase, carbamoyl phosphate synthetase 1, and ornithine transcarbamylase (OTC), are located in mitochondria, while the latter 3 enzymes, i.e., argininosuccinic acid synthetase, argininosuccinic acid lyase, and ARG1, are expressed in the cytosol [1]. ARG1, as the final

E-Mail karger@karger.com
www.karger.com/aha

Tanyel Zubarioglu
Division of Nutrition and Metabolism, Department of Pediatrics
Cerrahpasa Medical Faculty, Istanbul University, Kocamustafapasa Street, 34/E, Fatih
TR–34098 Istanbul (Turkey)
E-Mail tanyel0554@yahoo.com

Table 1. Clinical characteristics of argininemia patients

Patient No.	Age at diagnosis, years	Age, years	Gender	Consanguinity	Neonatal cholestasis	Major bleeding	Minor bleeding	Physical examination
1	7	13	Female	Yes	No	No	Yes (petechia)	Spasticity, mental retardation
2	2	7	Female	Yes	No	No	Yes (petechia)	Spasticity, mental retardation, epilepsy
3	4	12	Male	Yes	No	No	Yes (petechia, ecchymosis)	Spasticity, mental retardation
4	6	8	Female	Yes	No	No	No	Spasticity, mental retardation
5	5	7	Female	Yes	No	No	No	Spasticity, mental retardation
6	17	17	Male	Yes	No	No	No	Spasticity, mental retardation, epilepsy

step of the urea cycle, catalyzes the conversion of arginine to urea that is excreted in urine, and ornithine that participates in the cycle. ARG1 deficiency (ARG1D, OMIM #207800) is a very rare disorder, with a reported estimated incidence rate between 1/365,000 and 1/2,000,000 [1–4].

Different from all of other urea cycle disorders (UCD), neonatal or early infantile onset disease with hyperammonemic decompensation is rarely seen in ARG1D [1, 2, 5]. Classic clinical manifestations of the disease are spastic paraparesis, mental retardation, failure to thrive, and episodic hyperammonemia, mostly starting in early childhood [2, 6]. Uncommon presentations of ARG1D with neonatal cholestasis or cirrhosis have been reported [7, 8, 9]. Transient elevations of liver transaminases and coagulopathy have been reported during episodes of hyperammonemia, but a permanent coagulopathy has never been reported [1, 2, 6].

Here we present 6 ARG1D patients who were all affected by coagulation disturbances that were detected during routine investigations for hepatic involvement of argininemia.

Methods

All of the patients were followed up at the Pediatric Nutrition and Metabolic Disorders Clinic of the Cerrahpasa Medical Faculty in Istanbul, Turkey. Medical data were collected retrospectively from patients' charts. Data collection included: age at diagnosis, current age, gender, consanguinity, history of neonatal cholestasis, and major-minor bleeding history (Table 1).

The collected data included liver transaminases (alanine aminotransferase and aspartate aminotransferase), γ-glutamyl transferase, alkaline phosphatase, albumin, PT, INR, aPTT, and clotting factor levels. Clotting factor assays were performed as 1-stage clotting-based tests using commercial plasma deficient of the relevant factor on different coagulation analyzers (BCT, BCS of Dade-Behring/Siemens or CA-1500 of Sysmex) depending on the availability. Mutation analysis of the *ARG1* gene was performed by standard Sanger sequencing of single exons with flanking intronic regions. The laboratory results and the *ARG1* mutation analysis are shown in Table 2.

Results

Six patients with argininemia were enrolled into this study. One patient was diagnosed by family screening following the diagnosis of an affected sibling (patient 2). The remaining patients were diagnosed via a selective metabolic workup for neuromotor retardation. All of the patients had severe psychomotor retardation with spastic diplegia at the time of diagnosis. Patient 3 was also diagnosed with type 1 hyperlipidemia, with plasma triglyceride values ranging between 999 and 2,100 mg/dL. Molecular analysis of the *ARG1* gene was compatible with argininemia in 5 of the 6 patients, whereas patient 3 did not give consent for mutation analysis. All of the patients were routinely followed up for hepatic involvement due to ARG1D. Three patients (patients 1, 2, and 3) had minor bleeding findings (petechiae and ecchymosis) that were detected on routine physical examination, and 2 patients (patients 4 and 6) had abnormal laboratory findings noticed during preoperative testing. PT was prolonged and INR was increased, while aPTT was normal in all of the patients. Five patients had slightly elevated liver transaminases. Patient 4 had markedly elevated transaminases; a liver biopsy was performed but neither liver cirrhosis nor cholestasis was documented. Five of the 6 patients had low factor VII and factor IX levels, while other clotting factors were normal. Details of clinical characteristics and laboratory findings are summarized in Tables 1 and 2.

Table 2. Laboratory findings of argininemia patients

	Reference range	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Ammonia, μmol/L	11–60	18	23	46	43	31	48
PT, s	9.6–13.4	15.4–16.3	17.4–33.1	17.7–20	16.1–18.5	14.9–16.8	18.3
INR	0.9–1.2	1.39–1.48	1.48–3.48	1.53–1.73	1.48–2.21	1.37–1.62	1.46
aPTT, s	26–40.8	32.8–33.3	38–44.4	40.1–42.2	38.1–40.1	29.2–32.8	41.6
Platelet count, $n \times 10^3/mm^3$	100–300	135–154	199–227	258–303	114–176	233–264	237–270
Aspartate aminotransferase, IU/L	0–40	43–70	48–118	28–50	52–374	42–66	40–45
Alanine aminotransferase, IU/L	0–40	41–55	62–175	15–30	56–281	44–119	41–46
γ-Glutamyl transferase, IU/L	3–25	7–11	10–13	9–11	17–31	11–18	12
Alkaline phosphatase, IU/L	60–525	114–256	350–515	126–205	300–381	135–173	388
Albumin, g/dL	3.2–5.2	4.3	3.9	3.8	4.1	3.6	4.2
Total bilirubin, mg/dL	0.3–1.2	0.5	0.5	0.4	0.3	0.3	0.4
Direct bilirubin, mg/dL	0–0.2	0.2	0.2	0.1	0.2	0.2	0.1
Factor II, %	50–150	79.4	62.7	72	69		69.3
Factor V, %	50–140	73.6	85.7	64	147		64.5
Factor VII, %	50–150	35.5	3.2–16.6	26	30.2	57.3	49.5
Factor VIII, %	50–150	82.3	112.6	70	78.6		
Factor IX, %	50–150	32	19–29.8	38	17	67.3	36
Factor X, %	50–150	49.1	52.7	57	57.4	115.1	55
Mutation analyses		Exon 2: c.61C>T (p.Arg21*)	Exon 2: c.61C>T (p.Arg21*)	Not performed	Exon 2: c.61C>T (p.Arg21*)	Intron 1: c.58-3C>G (splicing)	Exon 4: c.446T>C (Leu149Pro)

Discussion

Argininemia due to ARG1D is the least common UCD, along with N-acetyl-glutamate synthase and CPS deficiencies. Unlike other UCD, hyperammonemia episodes and neonatal presentations are uncommon in argininemia. The classical presentation of the disease is characterized by progressive neurologic impairment in early childhood after an apparently normal development. The exact mechanism of the central nervous system injury is, however, still not fully understood. Direct toxic effects of elevated arginine and arginine metabolites including guanido components and nitric oxide have been suggested to be responsible for neurotoxicity [10, 11]. Besides the neurologic involvement, extraneurological symptoms are also present and the liver is the main affected organ. Hepatic involvement in argininemia can vary from mild hepatocellular injury with transient elevations of liver transaminases to coagulation abnormalities and hepatic failure as in other UCD [7–9, 12].

Here we present 6 patients with argininemia and coagulation disturbances. The coagulation abnormalities were detected during routine investigations for hepatic involvement of argininemia or before surgical procedures. All of our patients had mild to severe hepatic involvement with elevation of liver transaminases, and only 1 patient had mild hepatic fibrosis but no signs of hepatic failure were documented.

Data from the European registry of UCD show that highest frequency of liver disease is present in ASL and OTC deficiency among all UCD [13]. A recent study confirmed that neonatal histopathological changes in hepatic involvement of UCD are nonspecific, whereas older patients develop variable hepatic fibrosis and focal changes resembling glycogen storage disorder and cirrhosis [14]. The exact mechanism of liver injury in UCD is not known. It has been claimed that hyperammonemia can lead to a decrease in hepatic protein synthesis and especially to a reduction of coagulation factors, which are liver-derived proteins with a short plasma half-life [15]. According to this study, OTC-deficient patients were observed to have decreased factor VII and concomitantly increased INR during hyperammonemia episodes [15]. Although hyperammonemia is not a constant finding of argininemia, additional factors that have not been described yet might play a role in liver damage. A recent case report showed no correlation between the coagulopathy and plasma ammonia levels or with serum albumin levels, indicating the heterogeneous etiology of liver damage in OTC deficiency [16]. In this study, none of our patients had hyperammonemia accompanying the coagulation abnormalities. The coagulation abnormalities of our patients were per-

manent, independently of the patient's metabolic status. Although coagulation abnormalities in urea cycle disorders during hyperamonemia episodes have been well described, plasma ammonia levels were found to be within the normal ranges at the time of blood sampling in all of the patients. As a result, coagulation abnormalities were not found to be associated with hyperammonemic episodes. Therefore, we suppose that coagulation abnormalities are not correlated with hyperammonemia and additional mechanisms should play role in these patients.

Five of the 6 patients had low factor VII and factor IX levels, while other K vitamin-dependent factors like factors II and X were found to be normal. In order to rule out a vitamin K deficiency (factors VII and IX have the shortest half-lives among other vitamin K-dependent factors), parenteral vitamin K was attempted, with no improvement in coagulation parameters. Moreover, all of the patients had been on a low-protein diet with special formulas enriched with vitamin K_1 and none of them was nasogastrically/gastrically fed. Also, none of our patients was hospitalized for any medication before coagulation parameters were tested and none had history of chronic use of any medication that could interfere with vitamin K, such as antibiotics or anticonvulsants, except for patient 2, who had been using levetiracetam. Unfortunately, PIVKA-II levels were not measured for subclinical vitamin K deficiency.

Levels of factor V, which is synthesized only in liver and plasma albumin, were found to be within the normal reference ranges in all of the patients. As individual laboratory values which were accepted to be reliable indicators of synthetic liver function were obtained simultaneously with coagulation parameters and were found to be normal, it was thought that the factor VII and factor IX deficiencies in our patients most probably did not result from the hepatic involvement of arginase deficiency [17, 18]. Interestingly, patient 5 had impaired coagulation parameters but no additional single-factor deficiency was identified. In addition, the existence of both factor VII and factor IX, which was observed in 5 of the 6 patients, excluded the congenital factor deficiencies in our ARG1D patients.

Despite the high incidence of coagulation disturbances, none of our patients had severe life-threating hemorrhages and only patients 1, 2, and 3 had minor bleeding problems such as petechia and ecchymosis detected during a routine physical examination. Petechia and ecchymosis are signs of primary hemorrhagic problems, indicating that platelet associated or vascular problems might be present. Platelet counts and platelet morphologies were normal in our patients. As major bleeding symptoms were not observed in our patient group, advanced laboratory analyses to evaluate platelet functions were not performed.

In conclusion, we report the frequent observation of coagulation abnormalities in a small cohort of ARG1D patients and recommend investigating these patients for coagulation status even in the absence of an apparent liver dysfunction or major bleeding symptoms. Due to the rarity of argininemia, it is difficult to study this disease in large patient cohorts, which would be required to find out whether coagulation factor deficiencies are common findings in ARG1D and whether they are related to hyperammonemia or other signs of liver dysfunction. Further studies with large sample sizes and longer follow-up periods should elucidate the relationship and pathophysiological mechanisms of coagulopathy and argininemia.

Acknowledgement

The mutation analysis at Zurich University was supported by the Swiss National Science Foundation (grant 310030_153196 to J.H.).

Statement of Ethics

All of the procedures were performed in accordance with the ethical standards of the local Ethical Committee of the Cerrahpasa Medical Faculty and the Helsinki Declaration of 1975, revised in 2000. This study was approved by the local Ethical Committee of the Cerrahpasa Medical Faculty (No. 83045809-604.01.02).

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

Ertugrul Kiykim serves as the guarantor for this article. He accepts full responsibility for the work and/or the conduction of this study, had access to the data, and controlled the decision to publish. He was involved in conception, design, analysis, and interpretation of the data and in the drafting of this article. Tanyel Zubarioglu was involved in conception, design, analysis and interpretation of the data. Mehmet Serif Cansever was involved in analysis and interpretation of the data. Tiraje Celkan has was involved in interpretation of the data and critical revision this article for important intellectual content. Johannes Haberle was involved in analysis, interpretation of the data, and critical revision of this article for important intellectual content. Cigdem Aktuglu-Zeybek was involved in conception, design, analysis, and interpretation of the data.

References

1 Brusilow SW, Horwich AL. Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds; Childs B, Kinzler KW, Vogelstein B, associate editors. The metabolic and molecular bases of inherited disease. 8th ed. New York: McGraw-Hill; 2001, p. 1909-1963.

2 Scaglia F, Lee B. Clinical, biochemical, and molecular spectrum of hyperargininemia due to arginase I deficiency. Am J Med Genet C Semin Med Genet. 2006 May;142C(2 142C): 113–20.

3 Sparkes RS, Dizikes GJ, Klisak I, Grody WW, Mohandas T, Heinzmann C, et al. The gene for human liver arginase (ARG1) is assigned to chromosome band 6q23. Am J Hum Genet. 1986 Aug;39(2):186–93.

4 Summar ML, Koelker S, Freedenberg D, Le Mons C, Haberle J, Lee HS, et al.; European Registry and Network for Intoxication Type Metabolic Diseases (E-IMD). Electronic address: http://www.e-imd.org/en/index.phtml; Members of the Urea Cycle Disorders Consortium (UCDC). Electronic address: http://rarediseasesnetwork.epi.usf.edu/ucdc/. The incidence of urea cycle disorders. Mol Genet Metab. 2013 Sep-Oct;110(1-2):179–80.

5 Scholl-Bürgi S, Sigl SB, Häberle J, Haberlandt E, Rostásy K, Ertl C, et al. Amino acids in CSF and plasma in hyperammonaemic coma due to arginase1 deficiency. J Inherit Metab Dis. 2008 Dec;31(S2 Suppl 2):S323–8.

6 Crombez EA, Cederbaum SD. Hyperargininemia due to liver arginase deficiency. Mol Genet Metab. 2005 Mar;84(3):243–51.

7 Braga AC, Vilarinho L, Ferreira E, Rocha H. Hyperargininemia presenting as persistent neonatal jaundice and hepatic cirrhosis. J Pediatr Gastroenterol Nutr. 1997 Feb;24(2): 218–21.

8 Gomes Martins E, Santos Silva E, Vilarinho S, Saudubray JM, Vilarinho L. Neonatal cholestasis: an uncommon presentation of hyperargininemia. J Inherit Metab Dis. 2010 Dec; 33(S3 Suppl 3):S503–6.

9 Tsang JP, Poon WL, Luk HM, Fung CW, Ching CK, Mak CM, et al. Arginase deficiency with new phenotype and a novel mutation: contemporary summary. Pediatr Neurol. 2012 Oct;47(4):263–9.

10 Deignan JL, De Deyn PP, Cederbaum SD, Fuchshuber A, Roth B, Gsell W, et al. Guanidino compound levels in blood, cerebrospinal fluid, and post-mortem brain material of patients with argininemia. Mol Genet Metab. 2010;100 Suppl 1:S31–6.

11 Loscalzo J. An experiment of nature: genetic L-arginine deficiency and NO insufficiency. J Clin Invest. 2001 Sep;108(5):663–4.

12 Gallagher RC, Lam C, Wong D, Cederbaum S, Sokol RJ. Significant hepatic involvement in patients with ornithine transcarbamylase deficiency. J Pediatr. 2014 Apr;164(4):720–725.e6.

13 Kölker S, Valayannopoulos V, Burlina AB, Sykut-Cegielska J, Wijburg FA, Teles EL, et al. Erratum to: The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 2: the evolving clinical phenotype. J Inherit Metab Dis. 2015 Nov;38(6):1157–8.

14 Yaplito-Lee J, Chow CW, Boneh A. Histopathological findings in livers of patients with urea cycle disorders. Mol Genet Metab. 2013 Mar;108(3):161–5.

15 Laemmle A, Gallagher RC, Keogh A, Stricker T, Gautschi M, Nuoffer JM, Baumgartner MR, Häberle J: Frequency and pathophysiology of acute liver failure in ornithine transcarbamylase deficiency (OTCD). PLoS One 2016;12;11:e0153358.

16 Samuel N, Politansky AK, Hoffman R, Itzkovich S, Mandel H. Coagulopathy unmasking hepatic failure in a child with ornithine transcarbamylase deficiency. Isr Med Assoc J. 2013 Dec;15(12):777–9.

17 Bernuau J, Goudeau A, Poynard T, Dubois F, Lesage G, Yvonnet B, et al. Multivariate analysis of prognostic factors in fulminant hepatitis B. Hepatology. 1986 Jul-Aug;6(4):648–51.

18 Izumi S, Langley PG, Wendon J, Ellis AJ, Pernambuco RB, Hughes RD, et al. Coagulation factor V levels as a prognostic indicator in fulminant hepatic failure. Hepatology. 1996 Jun; 23(6):1507–11.